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March 31, 2005

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Certified by

Jon W. Dudas

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(b)(2).

INVENTOR(s)/APPLICANT(s)

Given Name (first and middle [if any])	Family Name or Surname	Residence (CITY AND EITHER STATE OR FOREIGN COUNTRY)
Allan L.	**GOLDSTEIN**	**Washington, D.C.**

☐ Additional inventors are being named on the ___ separately numbered sheets attached hereto.

TITLE OF THE INVENTION (280 characters max)

METHODS OF HEALING, TREATING AND/OR REVERSAL OF STENOSIS OR RESTENOSIS OF THE MYOCARDIUM AND CORONARY VESSELS, HEART VALVES AND SEPTA INJURIES OR DEFECTS USING THYMOSIN BETA 4, OR TB4 ANALOGUES, ISOFORMS, ANTIBODIES OR ANTI-SENSE PEPTIDES

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* [3]
☐ Drawing(s) *Number of Sheets* [ ]
☐ Application Data Sheet. See 37 CFR 1.76
☐ CD(s), Number _____
☐ Other *(specify)* _____

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

☒ Applicant claims small entity status. See 37 CFR 1.27
☐ A check or money order is enclosed to cover the filing fee
☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 02-2135
☐ Payment by credit card. Form PTO-2038 is attached.

Filing Fee Amount:

$80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE _____ Date 3-5-04

TYPED or PRINTED NAME **George R. Repper**
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REGISTRATION NO. **31,414**
Docket Number: **2600-111**

USE ONLY FOR FILING PROVISIONAL APPLICATION FOR PATENT

Methods of Healing, Treating and/or Reversal of Stenosis or Restenosis of the Myocardium and Coronary Vessels, Heart Valves and Septa Injuries or Defects Using Thymosin Beta 4, or TB4 Analogues, Isoforms, Antibodies or Anti-Sense Peptides

Technical Field of the Invention

The present invention relates generally to the prevention, healing and reversal of stenosis or restenosis that occur in the heart, heart valves and septa by the use of Thymosin B4 ($T\beta_4$) or its analogues, isoforms, or anti-sense peptides or antibodies which reduce inflammation, regulate actin, and induce angiogenesis.

Background

There are many causes of plaque and extracellular matrix build up in myocardial and coronary vessels, including but not limited to tissue damage, clotting abnormalities, vessel occlusion, defects or abnormalities and other such events. There are a number of compounds and procedures which when given within prescribed time frames may served to reduce or eliminate such occlusions, of often only temporarily, including angioplasty, mechanical devices such as coronary stents as well as pharmaceuticals and dietary modifications. Unfortunately, in many cases, the occlusive build-up of plaque and extracellular matrix molecules continues (restenosis) even after such procedures. It has been shown that $T\beta_4$ can induce angiogenesis and reduce inflammation in several rodent models. $T\beta_4$ also sequesters and regulates polymerization actin and stimulates collegen synthesis and other extracellular matrix molecules following wounding. However, there has been no known indication that such properties may be useful in treating occlusions and restenosis of coronary vessels and surrounding tissue such as heart valves. The ability to induce angiogenesis, decrease inflammation, and depolymerize actin could have a great potential in treating restenosis and similar indications in humans. Accordingly, there is a need in the art for additional factors and compositions that can reduce or prevent restenosis and occlusive build up in coronary vessels due to the build up of plaque, inflammation or other factors.

Summary of the Invention

The present invention is based on the discovery that $T\beta_4$ and other actin sequestering peptides and contain the actin binding motif and amino acid sequence LKKTET are chemotactic endothelial cells and can accelerate wound healing and modulate a number of key inflammatory cytokines e.g., IL-1BαIL-18 and chemokines such as MIP1-α_1 MIP-1β and MIP2.

As endothelial cells differentiate into capillaries or following any tissue injury, the gene for $T\beta_4$ is turned on and the levels of $T\beta_4$mRNA and $T\beta_4$ are elevated within the cells and surrounding tissues. The discovery that $T\beta_4$ could accelerate healing and can be covalently linked to fibrin and other extracellular molecules such as collagen led us to the

discovery that $T\beta_4$ and $T\beta_4$ analogues and other actin-sequestering molecules containing the amino acid motif LKKTET when covalently coupled to stents and/or administered during and after angioplasty can prevent or reverse restinosis and stenosis. $T\beta_4$ and other peptides containing the LKKTET motif also act as chemotactic and angiogenic factors for endothelial cells and thus potentially prevent or reduce the build up of plaque in coronary vessels and surrounding tissues.

A significant problem in chronic wounds and stents is the production of an over abundance of inflammatory cytokines in the injured tissue. $T\beta_4$'s ability to down regulate a number of key cytokines and chemokines and to accelerate the process of wound healing in normal and immunosuppressed animals may also be of importance in restenosis where inflammation, inflammatory intermediates and white cell infiltration have been implicated. In recently published studies of burn injuries to the eye, Sosne et al have clearly demonstrated a significant reduction in polymorponuclear leukocyte (PMN) infiltiation and clear reduction in a number of inflammatory cytokines.

The following are claimed:

1. The invention provides a method for the prevention, healing, or reduction of build up of plaque in coronary vessels and surrounding tissues, values and septa due to physiological insults, inflammation, cholesterol, or other factors which may occur in a subject in need of such treatment following stenting or angioplasty by the application of a therapeutically effective amount of a composition of an actin-sequestering, angiogenesis-inducing and anti-inflammatory polypeptide comprising the amino acid sequence LKKTET and conservative variants thereof having both anti-inflammatory properties, and actin depolymerization and angiogenesis activities.

2. Covalently or otherwise linking the claimed $T\beta_4$ and related molecules and other actin-sequestering peptides to stents or other medical devices or molecules used to prevent stenosis or restenosis.

3. Covalently or otherwise linking the claimed $T\beta_4$ and related molecules to fibrin-glue or similar compounds following coronary bypass surgery to prevent stenosis or restinosis.

4. Applying a therapeutically effective amount of the composition to a site on a periodic basis during a course of therapy to prevent or reduce stenosis or restenosis.

5. Administering $T\beta_4$ during and following angioplasty to prevent stenosis or restenosis.

6. Utilization of a composition which contains an agent that stimulates the production of LKKTET or $T\beta_4$ or some other actin-sequestering or angiogenesis inducing compound.

7. In one aspect of the method, the healing polypeptide is $T\beta_4$ or an isoform or oxidized form delivered in a tolerated dosage formula to reduce or prevent restenosis or other plaque build up.

8. The composition may be naturally derived, or produced using recombinant or synthetic methods.

9. A method of inducing angiogenesis, actin depolymerization or anti-inflammation in the subject tissue or organs in a subject, comprising administering to the subject a composition containing and agent which regulates an angiogenesis-inducing peptide, LKKTET, or $T\beta_4$ activity.

10. The method of claim 5, wherein the agent in an antibody.

11. The method of claim5, wherein the antibody is polyclonal.

12. The method of claim 5, wherein the antibody is monoclonal.

13. A method for ameliorating a coronary vessel or cardiac disorder associated with LKKTET, $T\beta_4$ comprising treating a subject having the disorder, at the site of the disorder or systemically, with an agent which regulates $T\beta_4$ activity.

14. The method of claim 9, wherein the $T\beta_4$ regulating agent is an antagonist of the $T\beta_4$ peptide.